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Association of interleukin-4 polymorphisms with multiple sclerosis in southeastern Iranian patients

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BACKGROUND AND OBJECTIVES: Immune system–related factors are important in the pathogenesis of multiple sclerosis (MS). Interleukin 4 (IL-4) as a helper T cell (2TH) cytokine is involved in the regulation of immune responses. Hence, this study was designed to explore the association between MS and polymorphisms in the -590 region of IL-4.

DESIGN AND SETTING: A descriptive study at Rafsanjan University of Medical Sciences, Rafsnajan from September 2009 to August 2010.

PATIENTS AND METHODS: Blood samples were collected from 100 MS patients and 150 healthy controls on EDTA precoated tubes. DNA was extracted and analyzed for IL-4 polymorphisms using restricted fragment length polymorphism in patients and controls. Demographic data were also collected by a questionnaire that was designed specifically for this study.

RESULTS: We observed a significant difference in the C/C, T/C, and T/T genotypes of the -590 region of IL-4 between patients with MS and healthy controls ($P<.001$).

CONCLUSIONS: We conclude that functional polymorphisms of IL-4 possibly play a crucial role in the pathogenesis of MS.

Multiple sclerosis (MS) is a sophisticated disorder in which the importance of immune system-related parameters in its pathogenesis is partially evident.[1] Both the loss of myelin and central nervous system (CNS) inflammation are frequent symptoms of MS.[2] The proper mechanisms responsible for myelin degeneration in MS have yet to be clarified;[2] however, recent published studies state that cognate immune system parameters are closely involved in the pathogenesis and complications of MS.[3] Accordingly, immune regulatory factors might influence the pathogenesis of MS.[3]

Cytokines are key factors in an appropriate immune response in autoimmune diseases[4,5] and interleukin 4 (IL-4), a prevailing helper T cell (2TH) cytokine in regulation of 1TH responses of the CNS.[6] Therefore, genetic factors that lead to a decreased cytokine expression enable the immune system to induce a vigorous immune response against CNS antigens in patients with MS.[7] The involvement of IL-4 in immunological disorders including MS,[7] systemic lupus erythematosus,[8] nephrotic syndrome,[9] graft rejection,[10] asthma,[11] and type 1 diabetes[12] is well documented. The human IL-4 gene is located on a cluster of cytokine genes on the q arm of chromosome 5 (5q31.1). It is approximately 10 kb in size with 4 exons and 3 introns (a and b). The IL-4 promoter region that extends to about 500 bp from the "TATA"-like sequence contains 5 polymorphic sites. The -590C/T single-nucleotide polymorphism is the most common IL-4 promoter olymorphic site (c). The paramount role of IL-4 as an inhibitory cytokine in autoimmunity and inflammation[13] explain the challenges of this cytokine on the pathogenesis of some diseases including MS. Previous studies revealed that IL-4 ex-

pression could be regulated by its polymorphisms in the -590 region.[6,14] Therefore, we aimed to investigate the polymorphisms in the -590 region of this cytokine in relapse-remitting multiple sclerosis (RRMS).

PATIENTS AND METHODS

Collection of samples

Specimens were collected from 100 relapsing-remitting patients with MS and 150 healthy controls during 2008–2009 in Rafsanjan University of Medical Sciences. The occurrence of MS was diagnosed by a neurologist according to clinical and paraclinical findings (magnetic resonance imaging study, oligoclonal bands in cerebrospinal fluid, and evoked potentials) based on the McDonald criteria.[15] Healthy control cases were also selected within the Rafsanjan population and matched by sex, age, and socioeconomic status following approval by Rafsanjan University of Medical Sciences Ethical Committee. Written consent forms were filled out by both patients and controls prior to specimen collection. The socioeconomic status was based on monthly income and education levels.

Table 1. Frequency of polymorphisms of IL-4 gene in multiple sclerosis patients and controls.

Condition Genotype (n, %)	Patients	Control	*P* value
C/C	76 (76%)	108 (72%)	<.001
T/C	23 (23%)	38 (25.3%)	
T/T	1 (1%)	4 (2.7%)	
Alleles			
C	175 (87.5%)	254 (84.6%)	<.001
T	25 (12.5%)	46 (15.4%)	

Table 2. Demographic and socioeconomic conditions of multiple sclerosis patient and controls.

Variant	Healthy control	MS patient	*P* value
Age	31 (8)	31 (6)	.85
Sex			
Female	69 (46%)	45 (45%)	.90
Male	81 (54%)	55 (55%)	
Socioeconomic status			
Weak	31 (21%)	22 (22%)	
Medium	72 (48%)	47 (47%)	.90
High	47 (31%)	31 (31%)	

DNA extraction and detection of polymorphisms

The peripheral blood was collected on EDTA precoated tubes and then genomic DNA was extracted by a commercial kit (Bioneer, South Korea). The extracted DNA samples were then stored at –20°C for further use.

IL-4 (-590C/T) gene polymorphism was analyzed by the polymerase chain reaction (PCR)-restricted fragment length polymorphism method. The sequences of primers were as follows: sense - 5'-TAAACTTGGGAGAACATGGT-3' and antisense - 5'-TGGGGAAAGATAGAGTAAT-3'. The PCR mixture was made up by addition of the following reagents to a 0.2 mL microcentrifuge tube on ice: 2.5 µL of Taq DNA polymerase buffer (10×), 0.5 µL of MgCl2 (stock concentration 1.5 mM), 0.5 µL of each dNTP [(dATP, dCTP, dGTP, and dTTP) stock concentration of 10 mM], 1 µL of each primer [(forward and reverse), stock concentration of 25 ng/µL], 1 µL of prepared DNA, and sterile double distilled water to a final volume of 25 µL. The PCR condition was an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of melting at 95°C for 50 seconds, annealing at 53°C for 50 seconds, and extension at 72°C for 40 seconds, with a final extension step of 5 minutes at 72°C using thermal cycler (C1000; Bio-Rad, USA). The PCR product of IL-4 (-590C/T) was a 195-bp fragment and was digested with AvaII into 175- and 20-bp fragments. The digested products were run on a 2.5% agarose gel (Cinnagen, Iran) and studied on Chemi-Doc model XRS (Bio-Rad) after staining with ethidium bromide. The assay yielded a single 195-bp band related to heterozygote C/T genotype; 2 bands of 20 and 175 bp related to the hemozygote CC genotype; and 3 bands of 20, 175, and 195 bp corresponded to homozygote TT genotype.

Statistical analysis

The Hardy-Weinberg equilibrium was assessed using genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. The statistical analysis of the differences between groups was determined by the chi-square test using EPI 2000 and SPSS software version 13 (IBM Corp., Armonk, NY USA). A *P* value less than .05 was considered as significant.

RESULTS

The evaluation of the polymorphisms in -590 of IL-4

by AvaII restriction enzyme showed that the prevalence of C/C genotype was 76 (76%) in patients with RRMS and 108 (72%) in controls. We found that the frequency of T/C genotype was 23 (23%) and 38 (25.3%) in patients with RRMS and controls, respectively. The frequency of T/T genotype in patients with RRMS was 1 (1%), whereas the frequency of this genotype in controls was 4 (2.7%) (**Table 1**). The statistical analysis of our data confirmed a significant difference between two groups in genotypes ($P<.001$). The frequency of the C allele was 175 (87.5%) and 254 (84.6%) in patients with RRMS and controls, respectively. Twenty-five (12.5%) cases of T allele were observed in patients, whereas the frequency of this allele was 46 (15.4%) in controls. To test for Hardy-Weinberg equilibrium, the chi-square in the patients group ($\chi^2=0.058$, $P=86.5$) and in the control ($\chi^2=0.036$, $P=88.5$) strongly indicated that both groups were in Hardy-Weinberg equilibrium. The statistical analysis of alleles indicated no significant difference between patients with RRMS and controls ($P= .374$) (**Table 1**). The calculated odds ratio of alleles was 0.79 for patients with RRMS and controls. There was no statistically significant difference between groups in mean age ($P=.85$), gender ($P=.90$), and or socioeconomic status of the participants ($P=.90$) (**Table 2**).

DISCUSSION

The pivotal role of the immune system in the etiology and pathogenesis of MS are well documented.[16-18] The crucial role of the cytokine network in the immune response is also documented.[4,19] It is well known that several factors—from infectious agents, hormonal conditions, to cytokine gene polymorphisms—control expression and secretion of cytokines.[19] The results of our study showed that there was a clear significant difference between control subjects and patients with RRMS in polymorphisms in the -590 region of IL-4. Previous studies have shown a close correlation between the C/C genotype and decreased expression of IL-4.[20,21] In this investigation, we observed that the C/C genotype is more frequent in patients with RRMS than controls. Therefore, based on our results, it seems that IL-4 gene polymorphisms can influence the pathogenesis of MS in our study population (south-eastern Iranian patients). In contrast to our results, Kamali et al showed that the polymorphisms in this region of IL-4 were not associated with MS in Shiraz city of Iran (a south-central part of Iran).[22] This discrepancy between our results and the findings of Kamali et al could be explained by the fact that some parts of the Shiraz population are mixed, with both Iranian and Turk ethnic groups, which differ in race and genetic constitution from our study population. Furthermore, Kamali et al studied IL-4 polymorphisms in all different phases of MS,[22] whereas all of our patients were in relapsing-remitting phases. Our results may reveal that IL-4 polymorphisms are more related to this type of disease rather than the other phases of MS.

These data may help clinicians to predict the beginning of the relapsing-remitting course of MS. Some studies have indicated that IL-4 -590 polymorphisms are associated with MS, which is consistent with out results.[6,7,23,24] For instance, Urcelay et al showed that these polymorphisms are associated with MS in the Spanish population.[6] An investigation in the Italian population also showed a significant correlation between IL-4 polymorphisms and late onset of MS.[23] The relationship between other polymorphisms in the IL-4 gene and MS also was reported by other investigators.[7,24] Therefore, it seems that IL-4 polymorphisms can significantly affect the expression of IL-4, and hence could presumably be involved in MS progression.

Overall, further studies are needed on patients with MS in different phases of MS with a wider sample size to differentiate between various MS phases according to the polymorphisms in Iranian patients with MS.

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